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Prevalence of Enteric Pathogens among International Travelers with Diarrhea Acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay)

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Stools from tourists from Europe and North America who acquired diarrhea in Mombasa (Kenya), Goa (India), or Montego Bay (Jamaica) were examined for enteric pathogens. Enterotoxigenic *Escherichia coli* (ETEC) was the most common pathogen (25%) identified in the 3 locations. Isolation of *Shigella* species was more frequent in Goa and Mombasa than in Montego Bay (10%, 9%, and 0.3%, respectively; $P < .005$). Viruses (rotaviruses and enteric adenoviruses) were found in 9% of travelers to the 3 areas. Of 275 ETEC isolates in this study, 158 (57%) produced a defined colonization factor antigen (CFA). Coli surface 6 (CS6) was the most frequent and was found in 41%–52% of CFA/CS-positive ETEC isolates. The frequency of resistance among bacterial enteropathogens to traditional antimicrobial agents was particularly high throughout the study period in all 3 regions. Quinolones were active against the bacterial enteropathogens in the 3 sites.

Travelers' diarrhea occurs after exposure to enteric pathogens during international relocation. Most episodes are self-limiting, and the causal pathogens usually do not cause persistent damage to the intestines. Travelers' diarrhea resembles endemic pediatric diarrhea in the host countries [1]. Both travelers and children are highly susceptible to the prevalent pathogens found in tropical developing countries as a result of environmental contamination, inadequate water supply, and poor sanitation and hygiene.

The prevalence of etiologic agents that cause travelers' diarrhea differs from area to area. For example, in Latin America, enterotoxigenic *Escherichia coli* (ETEC) is widely prevalent, with rates up to 40% [2, 3], and shows seasonal patterns in some areas [4]. In contrast, in Southeast Asia, ETEC less commonly (10%) [3] causes travelers' diarrhea. Other regional differences have been found among enteric pathogens isolated from travelers with diarrhea. Rotavirus infection is common in travelers to Mexico [5], and parasites have been commonly identified in travelers to Russia [6] and Nepal [7]. Information about the predominant organisms is critical in developing recommendations for management and to determine the potential value of enteric vaccines in the prevention of diarrhea. The present study was part of an international collaborative study of travelers' diarrhea designed to investigate the epidemiology, etiology, and socioeconomic impact of acute diarrhea among travelers visiting Mombasa (Kenya) [8], Goa (India) [8], or Montego Bay (Jamaica) [9]. Socioeconomic impact of travelers' diarrhea was analyzed and discussed in 2 associated publications [8, 9].

Methods

Setting. The study was conducted in 3 sites—Mombasa, Goa, and Montego Bay—between March 1996 and July 1998. In Goa, studies did not take place between May and September because of lack of tourism during the rainy season. The study population was primarily tourists from Europe and North America [8, 9].

Case definition. Classic diarrhea was defined in the study as the passage of ⩾3 unformed stools in 24 h plus the development of ⩾1 symptom of enteric infection (fever, abdominal pain or cramps, increased intestinal gas, nausea, vomiting, or passage of bloody stools). Moderate diarrhea was defined as passage of 1 or 2 unformed stools with ⩾1 additional enteric symptom or as passage of ⩾2 unformed stools without additional symptoms. Mild disease was defined as passage of 1 or 2 unformed stools without enteric symptoms.

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Written consent was obtained from each patient, and guidelines of the Committee for the Protection of Human Subjects, University of Texas Health Science Center at Houston, were followed.

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Recruitment. We placed advertisements at numerous hotels in Kenya, India, and Jamaica, and physicians were introduced to the travelers at welcome parties sponsored by the hotels. All stool samples were collected in hotel clinics established to evaluate and treat patients with diarrheal diseases. Staff from each selected local clinical laboratory received training in standardized methods. Travelers reporting with acute diarrhea within 48 h of onset who had not used antimicrobial agents in the past 7 days were invited to provide a stool sample. From Monday through Friday, stool samples went directly without transport medium to the project laboratory and were processed within 4 h. On weekends, transport medium (Culture & Sensitivity Media; Meridian Diagnostics) was used, and stools were processed on the Monday after collection.

Laboratory methods. Stool samples in cases of diarrhea were submitted to a local laboratory in each area, which was staffed by trained microbiology staff who used common procedures for etiology. Stools were examined for enteric protozoal parasites, including *Giardia lamblia, Cryptosporidia* species, and *Entamoeba histolytica,* by use of EIAs (Alexon). Adenoviruses and rotaviruses were detected by commercial ELISA kits (Premier Rotaclone and Adenoclone; Meridian Diagnostics). Cultures for enteric bacteria were completed by using 6 standard media: MacConkey, Tergitol, Hektoen enteric, *Yersinia,* TCBS, and *Campylobacter* agar plates. Five *E. coli* colonies were saved on peptone stabs for each patient for enterotoxin analysis in Houston. Heat-stable (ST) and heat-labile (LT) *E. coli* were detected by use of oligonucleotides labeled by T4 polynucleotide kinase and ^{32}P-ATP [10].

Various colonization factor antigens (CFAs) were characterized, as described elsewhere [11]. ETEC were first grown on CFA agar plates. After incubation at 37°C overnight, the expression of ETEC colonization factors was determined by dot blot test, using monoclonal antibodies specific for CFA/I, CFA/III, coli surface (CS) 1, CS3, CS4, CS5, and CS6. Isolates expressing CS3 alone or in conjunction with CS1 or CS2 were considered to be CFA/II. Isolates expressing CS6 alone or with CS4 or CS5 were considered to be CFA/IV. All bacterial isolates were identified by the API 20E system (bioMérieux Vitek). Antimicrobial susceptibility tests were done with bacterial pathogens, using the disk diffusion method of the National Committee for Clinical Laboratory Standards [12]. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 259213 were tested as control strains. The following antimicrobials (all supplied by BBL Microbiology Systems) were tested for organism susceptibility: ampicillin (Am-10), chloramphenicol (C-30), doxycycline (D-30), furazolidone (FX-100), gentamicin (GM-10), ofloxacin (OFX-5), streptomycin (S-10), sulfisoxazole (G-0.25), tetracycline (TE-30), trimethoprim-sulfamethoxazole (SXT), and trimethoprim (TMP-5).

Statistical analysis. Statistical analyses were done to define the significance of differences observed. Contingency tables were made containing information on the enteric pathogen, severity of illness, and CFA type in different sites. We used Fisher's exact test for computer analysis of data (Stata software, version 6.0) for different groups and pathogens. $P < .05$ was accepted as statistically significant.

Results

Pathogens identified in patients with diarrhea by the regions studied are shown in table 1. ETEC was the most common pathogen identified in the 3 locations. ETEC was significantly more common in Mombasa than in Goa (35% vs. 24%; $P = .0016$) or in Montego Bay (35% vs. 12%; $P < .0001$) and significantly more common in Goa than in Montego Bay (24% vs. 12%; $P < .001$). *Shigella* species pathogens were more frequently isolated in Goa and Mombasa than in Montego Bay (10%, 9%, and 0.3%, respectively; $P < .005$). In Montego Bay, stools were negative for an enteric pathogen more often than at the other study sites (68% vs. 45% and 47%, respectively; $P < .001$). Rotaviruses were found in 68 (6%) of 1079 patients with diarrhea and were the second most common enteropathogen in Montego Bay (8%). Mixed infections (infection with >1 enteric pathogen) were more common in Goa (11%) and Mombasa (6%) then in Montego Bay (5%). Isolation of *Shigella* species along with ETEC was the most common mixed infection in Goa, occurring in 7 patients, followed by *Salmonella* species with ETEC, occurring in 5 patients. Mixed infection of *Shigella* species and ETEC was the most common type in Mombasa (6 cases) followed by 3 cases of dual infection with *Vibrio* species and ETEC. *Campylobacter* species plus ETEC were found in 2 patients, and infection with *Salmonella* species and ETEC (2 cases) was identified in Montego Bay.

We pooled the data from the 3 sites to see whether there was a relationship between severity of diarrhea and infection by an enteric pathogen (table 2). There was no apparent increase in identification rates for enteropathogens in patients with more severe diarrhea. Although not included in the table, the pathogen detection rate in severe diarrhea cases (>6 unformed stools) in the 3 areas of study were similar: Goa, 60%; Mombasa, 57%; and Montego Bay, 51%.

Elsewhere, we described demographic characteristics of the subjects, including age, sex, country of origin, and sample collection time [8, 9]. Seasonal distribution of enteric pathogens

Table 1. Enteric pathogens identified in international travelers to Montego Bay, Jamaica; Goa, India; and Mombasa, Kenya.

Identified enteropathogen	Mombasa (n = 464)	Goa (n = 293)	Montego Bay (n = 322)	Total (N = 1079)
Aeromonas species	10 (2)	10 (3)	0	20 (2)
Campylobacter species	21 (5)	8 (3)	16 (5)	45 (4)
ETEC	164 (35)	73 (24)	38 (12)	275 (25)
Plesiomonas species	8 (2)	20 (7)	0	28 (3)
Salmonella species	13 (3)	30 (10)	25 (8)	68 (6)
Shigella species	40 (9)	30 (10)	1 (0.3)	71 (7)
Vibrio species[a]	16 (3)	16 (5)	1 (0.3)	33 (3)
Adenovirus	15 (3)	6 (2)	10 (3)	31 (3)
Rotavirus	26 (6)	16 (5)	26 (8)	68 (6)
Giardia lamblia	0	6 (2)	2 (0.6)	8 (0.7)
Entamoeba histolytica	0	14 (5)	2 (0.6)	16 (1)
Cryptosporidium species	0	6 (2)	1 (0.3)	7 (0.6)
Mixed infection	30 (6)	31 (11)	16 (5)	77 (7)
No pathogen detected	218 (47)	132 (45)	220 (68)	570 (53)

NOTE. Data are no. (%) of patients. EHEC, enterotoxigenic *Escherichia coli.*

[a] No *Vibrio cholera* 01 detected.

Table 2. Severity of diarrhea and pathogens isolated in Montego Bay, Jamaica; Goa, India; and Mombasa, Kenya.

Parameter	Mild or moderate nonclassic diarrhea (n = 97)	Classic illness: no. of unformed stools passed in 24 h before enrollment		
		3–5 (n = 334)	6–9 (n = 414)	⩾10 (n = 234)
Pathogen identified[a]				
Aeromonas species	1 (1)	4 (1)	9 (2)	6 (3)
Campylobacter species	0	12 (4)	14 (3)	15 (6)
ETEC[b]	38 (39)	73 (22)	103 (25)	58 (25)
Plesiomonas species	1 (1)	4 (1)	15 (4)	8 (3)
Salmonella species	4 (4)	17 (5)	26 (6)	22 (9)
Shigella species	7 (7)	14 (4)	23 (5)	17 (7)
Vibrio species	0	3 (0.8)	12 (3)	10 (4)
Adenovirus	3 (3)	7 (2)	9 (2)	12 (5)
Rotavirus	8 (8)	17 (5)	20 (5)	25 (11)
Giardia lamblia	0	4 (1)	3 (0.7)	1 (0.4)
Entamoeba histolytica	2 (2)	2 (0.6)	10 (2)	3 (1)
Cryptosporidium species	1 (1)	4 (1)	1 (0.2)	1 (0.4)
Subjects with pathogen identified	51 (53)	137 (41)	192 (46)	134 (57)
No pathogen detected	48 (49)	201 (60)	227 (55)	103 (44)

NOTE. Data are no. (%) of patients. ETEC, enterotoxigenic *Escherichia coli*.
[a] Some subjects had multiple infections.
[b] Three strains are missing from each ETEC classification.

identified in Montego Bay and Mombasa over the year of study was evaluated. Studies were suspended during 5 months of the year in Goa. Enteric bacterial infection showed a peak occurrence from February through April in Jamaica (range, 12%–40%), whereas rotaviruses were more common between October and March (range, 5%–8%). However, most cases of *Shigella* species infection were identified between May and July in Kenya (range, 26%–41%). ETEC did not show a seasonal trend in Montego Bay or Mombasa.

Table 3 shows the distributions of different enterotoxigenic phenotypes among ETEC isolated in the 3 areas. ST-only toxin was identified in 83 (51%) of 164 ETEC isolates from Mombasa visitors. LT-only was the most common toxin type detected in patients who visited Montego Bay (22 [58%] of 38 ETEC isolates identified). ST/LT production was the most common ETEC type seen in travelers to Goa (found in 33 [45%] of 73 ETEC isolates). CFAs were identified in 158 (57%) of the 275 ETEC strains studied.

As shown in table 3, the highest CFA frequency was observed among ST-only ETEC (found in 73 [63%] of all 115 ST-only ETEC), followed by ST/LT-producing strains (50 [56%] of 90) and LT-only producers (35 [50%] of 70 LT-only ETEC strains were CFA positive). The 2 most commonly identified CFA/CS types were CS6 (alone or with CS4 or CS5) and CS3 (alone or with CS1 or CS2). Of 110 CFA-positive ETEC strains in Mombasa, 53 (48%) were CS6 positive (alone or with CS4 or CS5). CS6 (alone or with CS4 or CS5) was identified in 9 (41%) of 22 CFA-positive ETEC strains in Goa. CS6 alone or with CS4 or CS5 was identified in 14 (54%) of 26 CFA-positive ETEC strains in Montego Bay. In Mombasa, 37 (34%) of 110 CFA-positive ETEC strains were CS3 positive (alone or in combination with CS1 or CS2). Eight (36%) of 22 CFA-positive ETEC strains isolated in Goa were CS3 positive (alone or with CS1 or CS2). In Montego Bay, 6 (23%) of 26 CFA-positive ETEC strains were CS3 positive (alone or with CS1 or CS2).

Antimicrobial susceptibility test results for the enteropathogens isolated are given in figure 1. The frequency of resistance to traditional antimicrobial agents was particularly high throughout the study period in all 3 regions. Multidrug resistance (resistance to ⩾3 antimicrobial agents) was demonstrated among 30%, 68%, and 69% of bacterial enteropathogens in Mombasa, Goa, and Montego Bay, respectively. The proportions of *Salmonella* species resistant to chloramphenicol, furazolidone, trimethoprim/sulfamethoxazole, trimethoprim, and tetracycline were significantly higher in Goa ($P < .05$, χ^2 test). Furazolidone and streptomycin resistance among *Shigella* isolates occurred most commonly in Goa. ETEC strains isolated from travelers to Goa were more resistant to ampicillin than those isolated at the other sites ($P < .05$, χ^2 test). Resistance frequencies were important at all 3 sites, without obvious differences for chloramphenicol (28%–62%), doxycycline (46%–57%), or trimethoprim (64%–79%). A low frequency of resistance to fluoroquinolone and ofloxacin (0.3%–5%) was found in all 3 regions.

Discussion

ETEC is the most common enteric pathogen infecting travelers after arrival in developing tropical countries [3]. In the present study, ETEC was found in 25% of the stool samples from patients with travelers' diarrhea in Mombasa and Goa but only

Table 3. Colonization factor antigen (CFA) types among enterotoxigenic *Escherichia coli* (ETEC) strains isolated from travelers to Mombasa, Kenya; Goa, India; and Montego Bay, Jamaica.

	Heat stable only			Heat labile only			Heat stable and heat labile		
CFA type	Mombasa	Goa	Montego Bay	Mombasa	Goa	Montego Bay	Mombasa	Goa	Montego Bay
ETEC	83	22	10	30	18	22	51	33	6
No CFA	25	15	2	14	12	9	15	24	1
Any CFA+	58	7	8	16	6	13	36	9	5
CFA/I	4 (7)[a]	0	1 (13)	0	0	3 (23)	0	1 (11)	1 (20)
CS1CS3	15 (26)	1 (14)	2 (25)	1 (6)	0	0	10 (28)	1 (11)	0
CS2CS3	1 (2)	1 (14)	0	0	2 (33)	1 (8)	8 (22)	2 (22)	0
CS3	0	1 (14)	0	0	0	1 (8)	2 (6)	0	2 (40)
CFA/III	1 (2)	0	0	3 (19)	1 (17)	0	0	2 (11)	0
CS4CS6	1 (2)	0	0	0	0	0	2 (6)	0	0
CS5CS6	2 (3)	0	0	0	1 (17)	1 (8)	0	1 (11)	0
CS6	30 (52)[b]	4 (57)	4 (50)	6 (38)[c]	1 (17)	7 (54)	12 (33)	2 (22)	2 (40)
Other CFA	4 (7)[d]	0	1 (13)[e]	6 (38)[f]	1 (17)[g]	0	2 (6)[h]	0	0

NOTE. Data are no. of strains; parenthetical values are percentages. CFA+, ETEC isolate tested positive for CFA; CS, coli surface.
[a] No. of any CFA+ isolates was used as a denominator in the category.
[b] One strain was positive for both CS6 and CFA/III.
[c] Three strains were positive for both CS6 and CFA/III.
[d] Putative colonization factor 166 (PCF166) (4 strains).
[e] PCF159 (1 strain).
[f] CS7 (3 strains), CS17 (3 strains).
[g] CS17 (1 strain).
[h] PCF159 (2 strains).

in 12% (38 isolates) in Montego Bay. Montego Bay is considered a moderate-risk area for travelers' diarrhea, and rates of bacterial infection would be expected to be lower than in high-risk areas of Latin America, Africa, and Southeast Asia.

ETEC may cause more than the 25% of occurrence that we found in the study areas. We previously used the multiplex polymerase chain reaction assay to help establish the importance of ETEC travelers' diarrhea [13]. In that study of travelers to Montego Bay, we found an ETEC rate double that found by use of the standard DNA hybridization procedure used in the present study. Undoubtedly, an important amount of otherwise pathogen-undetected diarrhea in high-risk areas is caused by ETEC. In the present study, ETEC did not show seasonal occurrence patterns in Jamaica and Kenya, which agrees with a previous study of pathogens in travelers' diarrhea in Jamaica [9]. A seasonal pattern of ETEC infection has been seen in other areas, including Mexico [4] and Morocco [14].

In a separate study, we looked at the prevalence of enteroaggregative *E. coli* as a cause of travelers' diarrhea by using the HEp-2 cell assay [15] in 3 populations: in Goa (the same population included in this study); Ocho Rios, Jamaica (a region of Jamaica different from that in this study); and Guadalajara, Mexico. Enteroaggregative *E. coli* was identified in 26% of the travelers' diarrhea cases and was second to ETEC as the most common enteropathogen identified in world studies.

In the present study, non-ETEC bacterial enteric pathogens (*Salmonella, Shigella, Campylobacter, Aeromonas,* and *Vibrio* species) were isolated from 265 (25%) of 1079 patients, and *G. lamblia, E. histolytica,* and *Cryptosporidia* were identified in 31 (3%) of 1079 and rotaviruses and adenoviruses in 99 (9%) of 1079 patients with diarrhea. In Montego Bay, rotaviruses were the second most common enteric pathogen in persons with travelers' diarrhea (8% of cases vs. 12% of ETEC). The fact that there were similar detection rates in classic mild and moderate travelers' diarrhea suggests that the 3 degrees of travelers' diarrhea are different clinical expressions of the same enteric infection. Mixed infections were encountered in 5%–11% of patients in the study. The distribution of pathogens in mixed infections appeared to reflect the relative importance of individual etiologic agents in the area studied. There did not appear to be a special relationship between pathogens.

Because we do not have total traveler numbers for each site, it was not possible to calculate the incidence of travelers' diarrhea in the regions. In the present study, we found no relationship between intensity and severity of clinical illness in patients with enteric symptoms according to the presence or absence of a specific enteric infection. The percentage of subjects with enteric infection by a specific enteropathogen, including ETEC with its variation in toxin types, *Salmonella* species, and *Shigella* species, did not differ between those with mild or moderate nonclassic diarrhea and variable degrees of severity of classic travelers' diarrhea. This suggests that travelers with mild enteric symptoms are as commonly infected with an enteric pathogen as those with more intense diarrheal illness.

Other studies have shown that a natural ETEC immunity occurs as people remain at risk for infection [16]. This finding has given researchers encouragement that a protective ETEC vaccine might be developed, and a number of ETEC vaccine candidates are currently under development. It has been concluded that a human ETEC vaccine should be given orally to evoke both

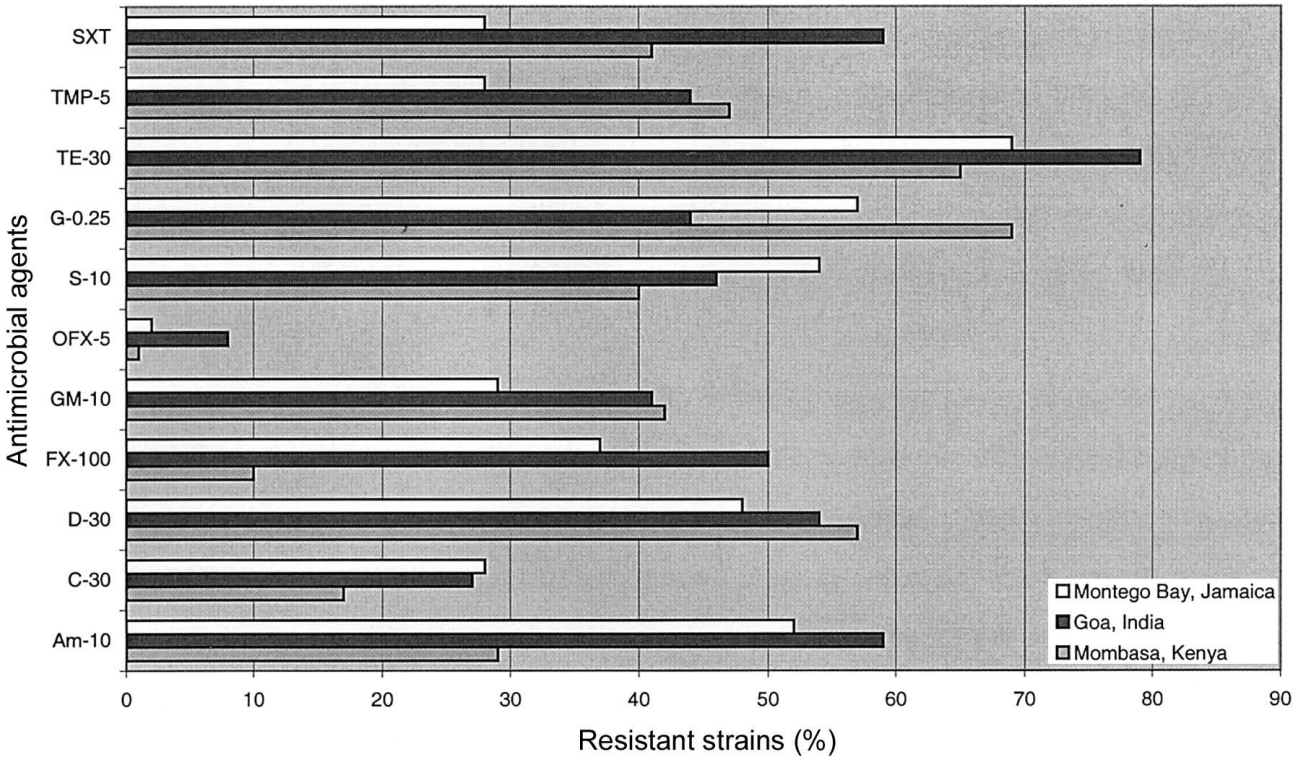

Figure 1. Proportion (%) of isolates resistant to antimicrobials, by geographic area. In total, 246 isolates were tested by the disk diffusion method for antimicrobial susceptibility. SXT, trimethoprim-sulfamethoxazole; TMP-5, trimethoprim; TE-30, tetracycline; G-0.25, sulfisoxazole; S-10; streptomycin; OFX-5, ofloxacin; GM-10, gentamicin; FX-100, furazolidone; D-30, doxycycline; C-30, chloramphenicol; and Am-10, ampicillin.

anticolonization and antitoxic immune responses locally in the gut [17]. The present study provides information that may be useful when the ideal approach for immunologic control of travelers' diarrhea is considered. At least one of the most promising vaccine candidates uses cholera toxin B-subunit [18], which is immunologically and physiologically related to LT of ETEC. Anti-LT immunity does not protect against ETEC producing ST only, and ST is not immunogenic unless coupled to a carrier [19]. It has not been possible to synthesize ST toxoids that induce a good neutralizing antibody response without residual toxicity [19].

Of 275 ETEC isolates seen in this study, 115 (42%) produced ST only and would not be expected to be prevented by an anti-LT vaccine. ETEC expressing LT alone was the most common enterotoxin type found in Montego Bay (58%). To be effective, vaccine candidates should probably contain a number of ETEC CFAs. In all, 158 (57%) of 275 ETEC isolates in the present study produced defined CFAs. This is a higher CFA rate than that seen in studies in South America, including Argentina [11] and Chile [20]. In the study in Chile [20], a CFA was found in 23% of ETEC isolates. CFA/II was the principal adhesin type among ETEC in 2 South America countries [11, 20], and a study in Peru demonstrated the importance of CFA/IV [21]. For a vaccine designed for Mombasa, Goa, and Montego Bay, based on findings in the present study, the preparation should optimally include CFA/II (CS3 is the most important) and CFA/IV (CS6 is the most important) components. CS6 was commonly identified in ST-only ETEC producers in the present study (52% in Mombasa, 57% in Goa, and 50% in Montego Bay). Thus, on the basis of our results, we believe that an ETEC vaccine for widespread use in developing countries should also include CS6 and CS3. A vaccine candidate currently under development contains a combination of CTB and CFA/I and CS1-CS5 on inactivated bacteria. In the present study, 15% of ETEC isolated from all sites were LT negative and did not produce detectable CFAs. Most LT-negative, CFA-negative ETEC isolates were from Mombasa and Goa (15% and 21%, respectively), and 5% were from Montego Bay. An LT/CFA vaccine would not be expected to protect against this proportion of ETEC diarrhea and obviously not against other non-ETEC enteric pathogens.

Trimethoprim resistance is widespread among bacterial enteropathogens worldwide, rendering this drug no longer effective for managing travelers' diarrhea. ETEC, *Salmonella* species, and *Shigella* species isolated from 3 areas in the present study showed high-level resistance to trimethoprim and trimethoprim/sulfamethoxazole (51%), ampicillin (50%), doxycycline (54%), and gentamicin (39%). We have no explanation for the lower rate of multiantimicrobial resistance found in enteric pathogen

isolates in Mombasa. Although resistance to fluoroquinolones has been reported among *Salmonella typhi* in India [22], and resistance to fluoroquinolones among *Campylobacter* isolates in Thailand [23] and Spain [24] has been reported, fluoroquinolones remained active in vitro against bacterial enteropathogens causing travelers' diarrhea in the 3 areas that we studied. On the basis of our findings, we believe that drugs of this class should be considered to be the drugs of choice for treatment of travelers' diarrhea in adults in most regions of the world. For travel to areas where fluoroquinolone-resistant pathogens are common (e.g., Thailand), azithromycin may be the preferred antimicrobial therapy [23].

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